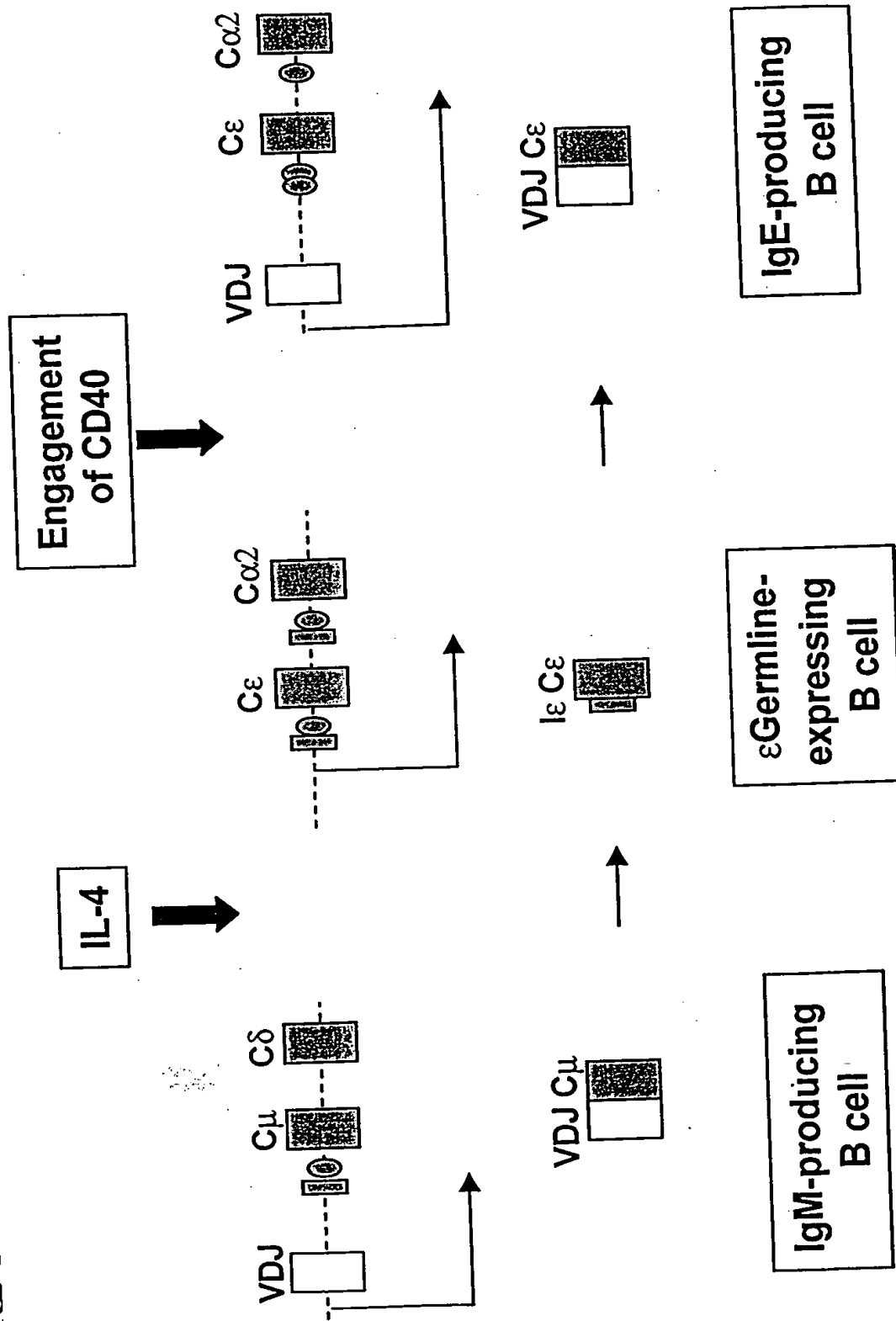


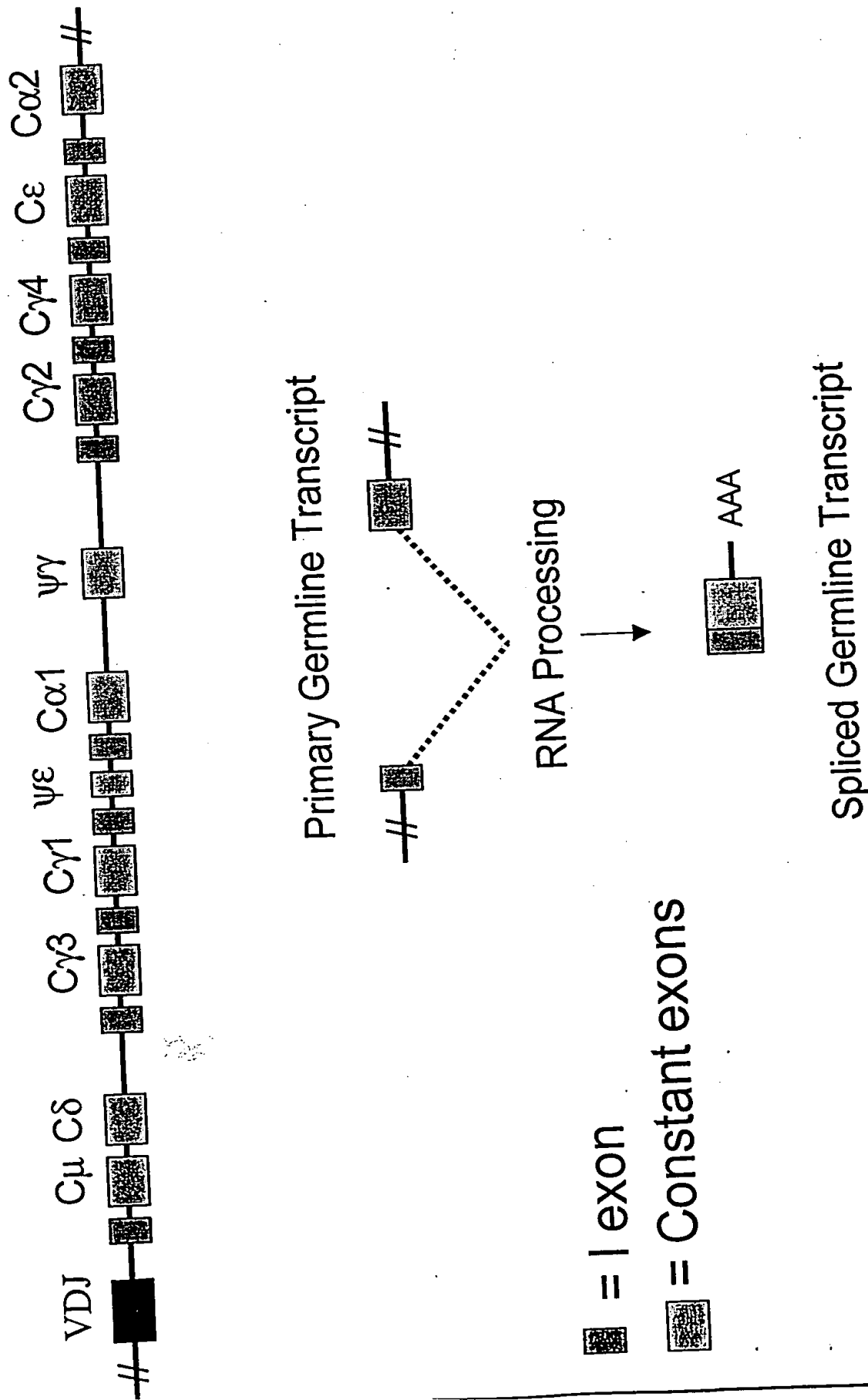
ϵ Germline Transcription and IgE Switching

FIGURE 1



Chromosome 14 Human Heavy Chain Gene Map

FIGURE 2



Sequences of RPA Probes for Human Immunoglobulin
Germline Transcripts

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCCACTCAGCACTGCGGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCACGTGAAGCACTACACGAATCCCA
GCCAGGATGTGACTGTGCCCTGCCCACT
TCCCCCACCTCCCCCATGCTGCCACCCCCGACTGTCGCTGCACCGACCGGCCC

Germline Ig Epsilon Probe

GGCTCCACTGCCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCGGCAGCATACCGGAGCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCCCATCGGTCTTCCCCC
TGGCACCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAGGCGCCCTGA
CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCC
CAAATCTTGTGACAAAACCTCACACATGCCCCACCG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGGCCCCAGGTGAACGGAGGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCGTG
GAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGT
AGATCACAAGCCCAGCAACACCAAGGTG
GACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCAACCGTGCCCAAGCAC
CACCTGTGGCAGGACCGTCA

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCCATCGGTCTTCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAATCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACACCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCT
CAAAACCCCACTTGGTGACACAACTCACACATGCCCAACGGTGCCCAAGAGCCC
AAATCTTGTGACACACCTCCCCCGTGCC
CACGGTGCCC

FIGURE 3
3 OF 3

Germline Ig Gamma 4 Probe

GGCCAGCACCA.CATGGAAGCCCAAGCGGAGCCAGCACGGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCAACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGGAGAGGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGGCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGGTGACGGTGTTCGTGGAACCTCAGGCGCCCTGACCAGE
GGCGTGACACCTTCCCGGCTGTCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
CTTGGGCACGAAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA
TATGGTCCCCCGTC

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Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-1 Probe

GGCCTGGGCGGGGCCAGGGCTCCCTCCCCACAGCAGCAGCCTCTTGGCAGGCAG
CCAGACGCCCCGTGAGGGTGGACCTGCCA
TGAGGGCCTGCACGCGGAGGCCGCCACTCAGCACTGCGGGCCCTCCAGCA
GCTGACCAGCATCCCCGACCAGCCCCA
AGGTCCTTCCCGCTGAGCCTCTGCAGCACCCAGCCAGATGGGAACGTGGTCAT
CGCCTGCCTGGTCCAGGGCTTCTTCCCC
CAGGAGCCACTCAGTGTGACCTGGAGCGAAAGCGGACAGGGCGTGACCGCC
AGAAACTTCCCACCCAGCCAGGATGCCTC
CGGGGACCTGTACACCAAGAGCAGCCAGCTGACCCCTGCGGCCACACAGTGC
CTAGCCGGCAAGTCCGTGACATGCCAC

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGCGCCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCTGCACGCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCTACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCAC

Germline Ig Epsilon Probe

GGCTCCACTGCCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGOCACACCATCCACAGGCACCAAATGGACGACCGGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCCTTG
ACCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCTGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGGCCCCAGGTG
AACGGAGGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGTCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCATCGGTCTTCCCCC
TGGCACCCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTCCCCGAACCGG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACAACAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGGCCCCAGGTGAACGGAGGGGGCTTGTCCAGTCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGG

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGGCCCCAGGTG
AACCGAGGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGTCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTCCCCGAACCGGTG
ACGGTGTCTGGAACTCAG

Germline Ig Gamma 4 Probe

GGCCAGCACCATGGAAGCCCAAGCGGAGCCAGCAOJGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGACAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGAGACGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGACCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCTGGTCAAGGA
CTACTTCCCCGAACCGG

FIGURE 4

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FIGURE 5

RPA PROBES

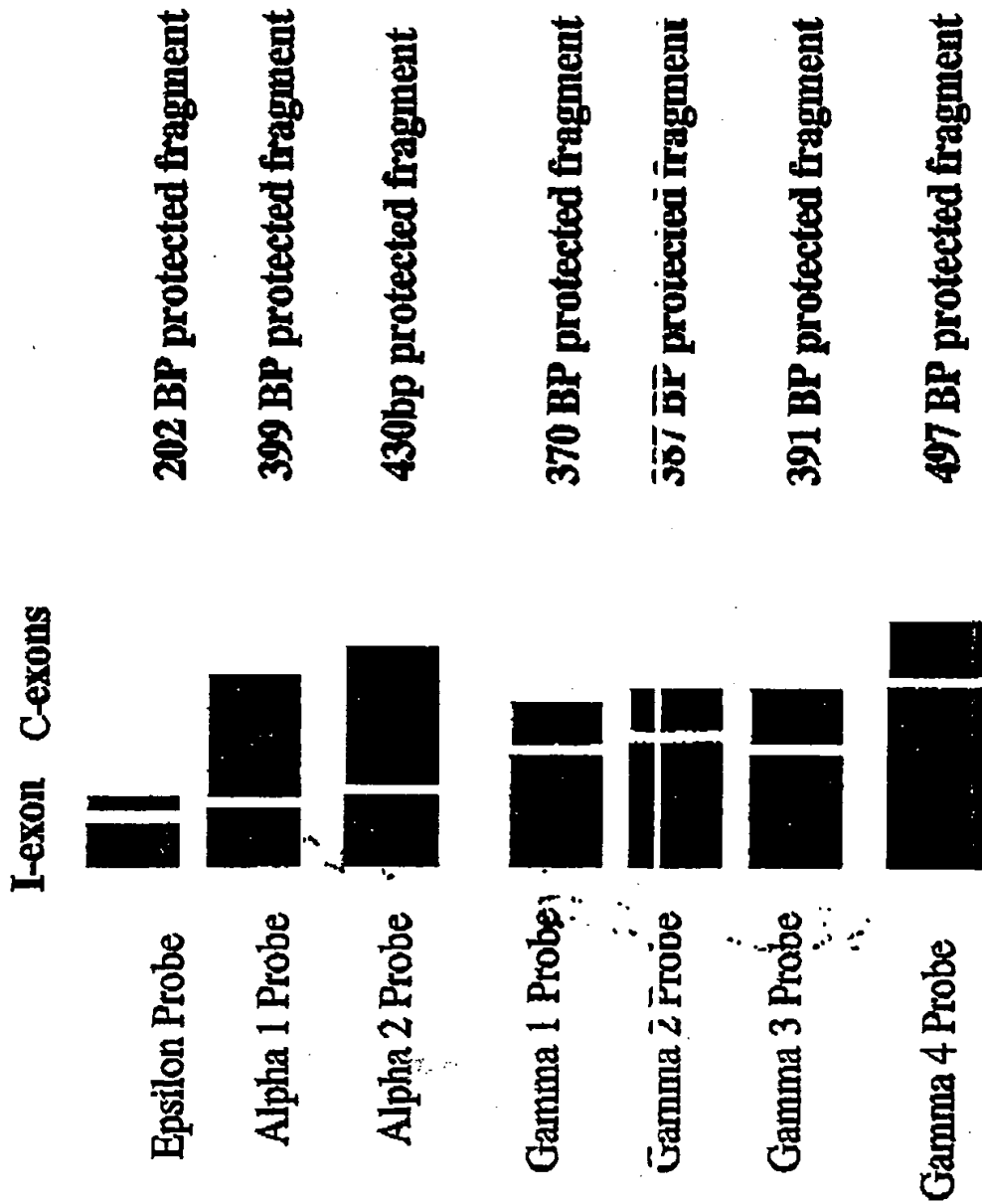
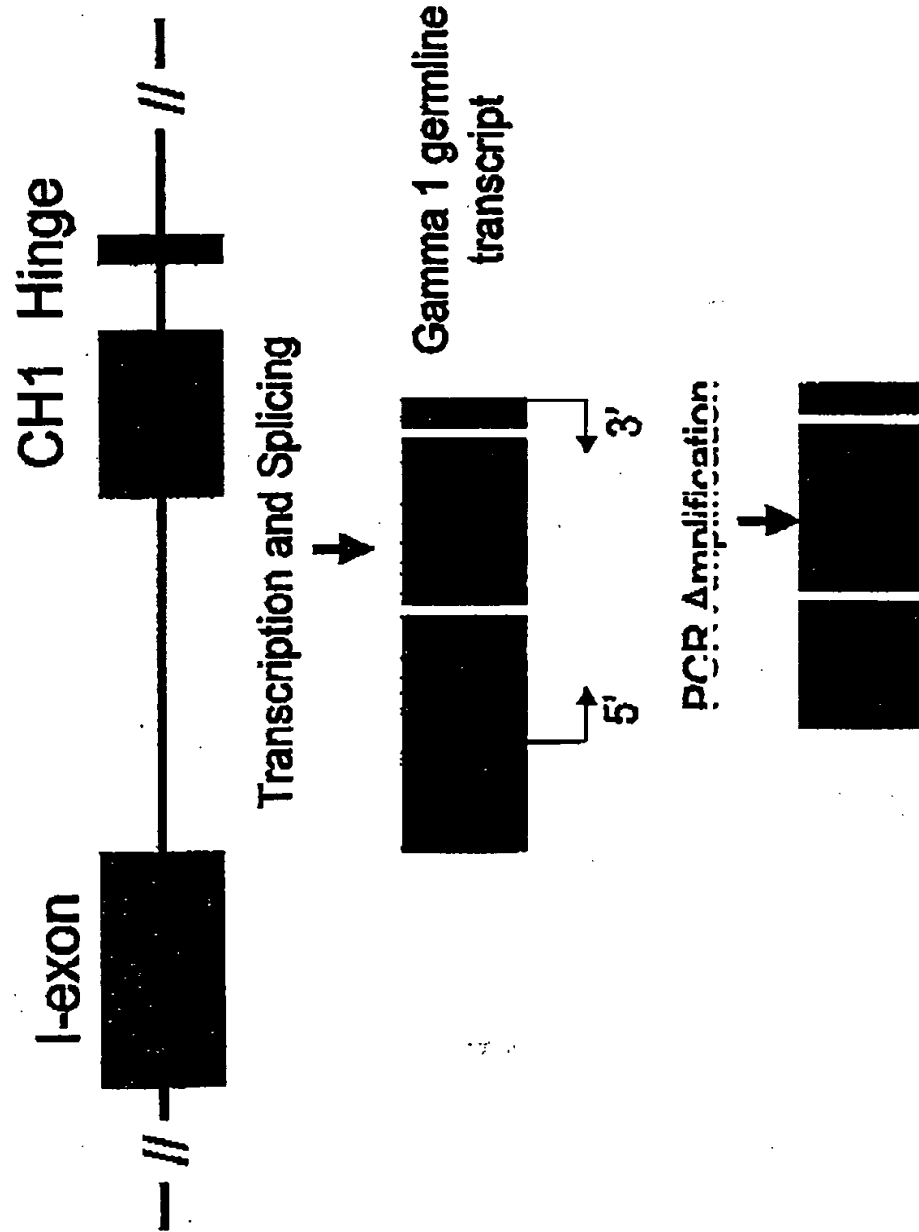


FIGURE 6

102501 05641360

Gamma 1 Probe



The Gamma 1 5' and 3' Primers amplified a completed probe of 370 BP

RNAse Probe Protection Assay

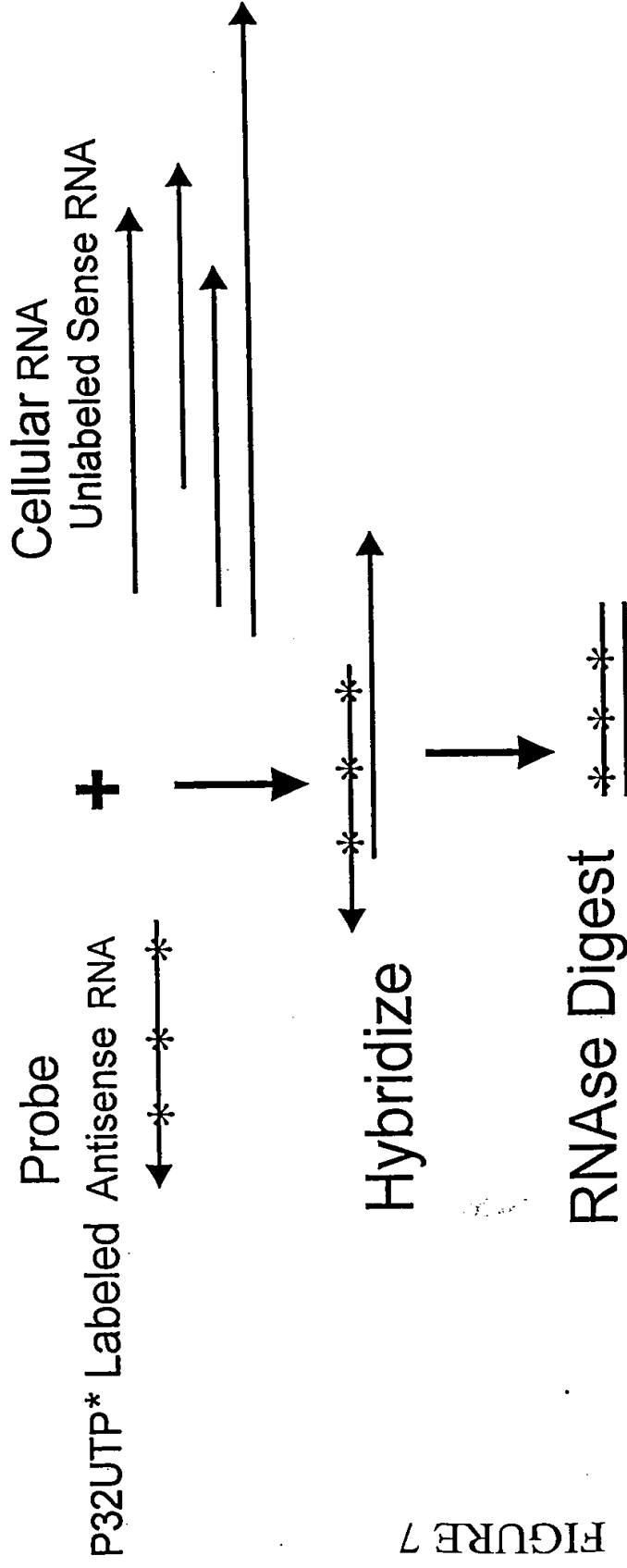
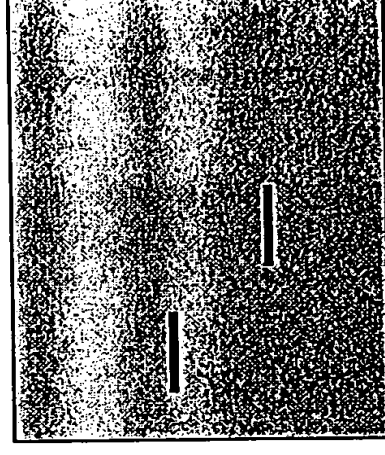


FIGURE 7

Run undigested probe vs digested protected fragment on acrylamide-Urea gel



Visualize using beta imaging equipment

FIGURE 8

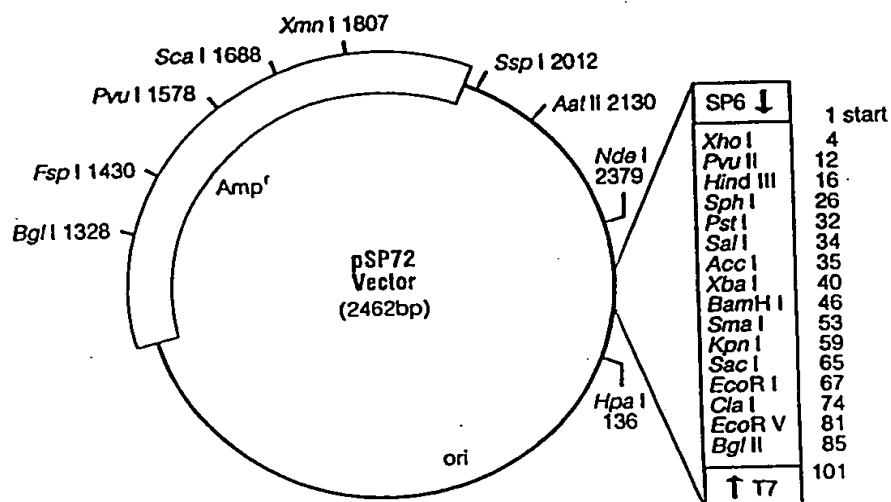


Figure 2. pSP72 Vector circle map and sequence reference points.

1. Sequence reference points:

a. SP6 RNA polymerase transcription Initiation site	1
b. T7 RNA polymerase transcription Initiation site	101
c. SP6 RNA polymerase promoter	2446-6
d. T7 RNA polymerase promoter	96-118
e. multiple cloning sites	4-90
f. β -lactamase (Amp ^r) coding region	1135-1995
2. Specialized application:
 - a. transcription *in vitro* from dual opposed promoters (For protocol information, please request Promega's Riboprobe® *in vitro* Transcription Systems Technical Manual, #TM016.)
3. The pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.
4. Blue/white screening for recombinants is not possible with the pSP72 Vector.

FIGURE 9

[illegible]

Alpha - 2

Epsilon

Gamma - 1

Gamma - 2

Gamma - 3

Gamma - 4

X56796 = I Region Exon
K01316 = Constant Region Exon